The Influence of Cobra-venom on the Proteid Metabolism.

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Although so much valuable work has been done upon the physiological action of Cobra-venom by Fraser, Calmette, Elliot and others,* so far no observations of its effects upon the metabolism have been recorded. While its peculiarly selective action on the central nervous system would seem to suggest the absence of any marked general action, the demonstration afforded by Elliot's work of its influence and the way in which peripheral nerve mechanism may be attacked, and of its interference with respiration, and the direct local action on the tissues into which it is injected, seem to indicate that its toxic action may extend to protoplasm generally and that it may thus lead to modification of the proteid metabolism, whether in the direction of altering the rate of proteid metabolism or of modifying the synthetic processes in the liver by which urea is elaborated, as do the toxins of certain micro-organisms.

I have to thank Sir Thomas R. Fraser for his kindness in giving me the Cobra-venom; and also to thank Dr. Noël Paton for much valuable assistance in this investigation.

General Plan of Investigation and Methods.

Dogs were used for this investigation. Before each experiment they were fed for some days on a fixed diet of oatmeal porridge and milk in order to establish nitrogenous equilibrium. The urine was collected by keeping the animal in a cage with a sloping bottom made of zinc under which a suitable vessel was placed. The floor was kept scrupulously clean, and fæces were removed as soon as possible after they were passed. The urine was collected daily at 10 A.M.

When the animal came into nitrogenous equilibrium, sub-lethal and in some cases lethal doses of Cobra-venom were injected subcutaneously.

Urine.—The reaction and specific gravity were taken. The quantity collected was noted and the urine diluted to a convenient volume. The amounts of the following ingredients were determined by the methods

* See Fayrer: 'Thanatophidia of India'; Brunton and Fayrer, 'Roy. Soc. Proc.,' vol. 22, 1874; Wall, 'Indian Snake Poisons,' 1883; Nicholson, 'Ophiology,' 1893.

enumerated, duplicate analyses were made in all cases and the mean of these taken :—

1. Total nitrogen by Argutinsky's modification of Kjeldahl's method.
2. Nitrogen in urea by Bohland's method by precipitating with phospho-tungstic (Merck's) and hydrochloric acids.
3. Nitrogen of ammonia by Schlössing's method.
4. Nitrogen not in urea (non-urea nitrogen) was calculated by difference.
5. Nitrogen in purin bodies by Krüger and Wulff's method.*
6. Phosphoric acid as P_2O_5 by titrating with uranium nitrate.
7. The percentages of nitrogen in urea, not in urea (non-urea nitrogen), in ammonia, in purin bodies, in other compounds were calculated in terms of total nitrogen.
8. The percentages of P_2O_5 were calculated in terms of total nitrogen.

Averages of the figures before and after the injections were determined.

Albumin was tested for by cold nitric acid and by heat and acetic acid.

Some of the experiments were rendered valueless by the early development of an abscess at the seat of inoculation, but the following were carried on, without disturbance from such accidents.

In all the experiments no marked symptoms were produced. In some cases after injection the dog lay very quiet for a few hours. In a few there was a local swelling observed next day, but it soon disappeared.

Experiment 1.

For this experiment a retriever weighing 18 kilogrammes was used. The dog was kept in its cage for two or three days before the examination of the urine was begun, so that it might get accustomed to its diet and nitrogenous equilibrium be established.

Food Analyses.

Analyses of the food given to the dog showed the amount of nitrogen to be :—

Daily diet: 500 c.c. milk.....	= 1·83 grammes nitrogen	
200 grammes oatmeal	= 4·30	„
Total intake of nitrogen per diem...	= 6·13	„

Analyses of the urine were begun on November 16, 1903, and on the 19th, at 4 P.M., 1·25 milligrammes Cobra-venom dissolved in 0·5 c.c. sterilised normal saline solution was injected subcutaneously in the side.

* *Cf.* 'Zeit. für Phys. Chem.,' vol. 20, p. 177, 1895.

Table

Date.	Quantity of urine in c.c.	Sp. gr.	Reaction.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.
1903—									
November 16 ...	380 diluted to 500	1023	acid	1·098	5·45	0·882	4·11	0·216	1·36
„ 17 ...	315 to 500	1018	acid	0·734	3·67	0·566	2·83	0·168	0·84
„ 18 ...	380 to 500	1020	acid	0·927	4·63	0·759	3·79	0·168	0·84
									1·25 milligrammes Cobra
November 19	500	1021	acid	1·210	6·05	0·959	4·79	0·251	1·26
„ 20	500	1015	acid	0·820	4·102	0·622	3·11	0·198	0·99
„ 21 ...	385 to 500	1019	acid	0·811	4·053	0·603	3·01	0·208	1·04
„ 22 ...	435 to 500	1021	acid	0·977	4·89	0·829	4·14	0·148	0·75
„ 23	500	1021	acid	1·064	5·32	0·874	4·37	0·190	0·95
„ 24 ...	430 to 500	1015	acid	0·692	3·458	0·573	2·86	0·176	0·88
									2·50 milligrammes Cobra
November 25	500	1015	acid	0·778	3·892	0·634	3·17	0·144	0·72
„ 26	600	1015	acid	0·746	4·477	0·547	3·29	0·197	0·98
„ 27	700	1015	acid	0·697	4·609	0·566	3·96	0·131	0·64
„ 28	500	1015	acid	0·784	3·920	0·591	2·95	0·193	0·97
„ 29 ...	250 to 500	1016	slightly alkaline	0·3738	1·869	0·2702	1·35	0·1036	0·51
„ 30	600	1015	acid	0·7098	4·259	0·585	3·51	0·1286	0·74
									Averages Before and
November 16, 17, and 18 (before injection)				0·919	4·59	0·736	3·58	0·184	1·01
„ 19, 20, 21, and 22 (after 1st injection).				0·954	4·77	0·753	3·76	0·201	1·01
„ 25, 26, 27 and 28 („ 2nd „).				0·751	4·22	0·585	3·34	0·166	0·83

I.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other compounds.	P_2O_5.
0·0728	0·364	0·0078	0·0390	0·281	1·405	80	20	6·6	0·71	12·69	26
0·0301	0·105	0·0097	0·0485	0·147	0·735	77	23	4·1	1·32	17·58	20
0·056	0·280	0·0047	0·0238	0·221	1·105	82	18	6·04	0·51	11·45	24
venom injected at 4 P.M.											
0·0889	0·4445	0·0092	0·0462	0·377	1·885	88	12	7·34	0·76	3·90	31
0·0497	0·2485	0·0098	0·0490	0·213	1·065	76	24	6·06	1·20	16·74	26
0·0596	0·2982	0·0154	0·0770	0·232	1·160	74	26	7·35	1·90	16·75	28·6
0·0902	0·4508	0·0084	0·0420	0·299	1·495	85	15	9·22	0·86	4·92	30·6
0·0714	0·3570	0·0087	0·0434	0·357	1·785	82	18	6·71	0·82	10·45	33
0·0560	0·2800	0·0073	0·0364	0·191	0·955	83	17	8·23	1·05	7·72	27·6
venom injected at 9.30 A.M.											
0·0612	0·3059	0·0094	0·0469	0·181	0·905	82	18	8·0	1·20	8·80	23·3
0·0938	0·5628	0·0070	0·0420	0·227	1·362	75	25	12·4	0·94	11·66	30·4
0·1008	0·7056	0·0077	0·0539	0·235	1·645	81	19	14·4	1·15	3·45	33·7
0·0916	0·4578	0·0104	0·0518	0·203	1·015	75	25	11·7	1·34	11·96	26
0·0885	0·4424	0·0062	0·0308	0·076	0·380	72	28	10·4	1·45	16·15	18
0·0641	0·3847	0·0073	0·0437	0·223	1·338	82	18	9·03	1·02	7·95	31
After the Injection.											
0·053	0·249	0·0074	0·0371	0·216	1·08	80	20	5·58	0·85	13·91	23
0·072	0·360	0·0107	0·0586	0·280	1·40	80	20	7·99	1·18	10·58	29
0·062	0·508	0·0084	0·0511	0·236	1·23	78	22	11·6	1·16	8·97	28

Table II.—Weight

Date.	Quantity of urine in c.c.	Sp. gr.	Reaction.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.
1904—									
March 22.....	750 diluted to 1000	1015	acid	0·552	5·52	0·455	4·55	0·097	0·97
„ 23.....	710 to 1000	1014	acid	0·504	5·04	0·434	4·34	0·070	0·70
„ 24.....	550 to 1000	1020	acid	0·585	5·85	0·490	4·90	0·095	0·95
„ 25.....	550 to 1000	1019	acid	0·543	5·43	0·465	4·65	0·078	0·78
									5 milligrammes Cobra
March 26.....	265 to 500	dark brown 1027	strongly acid	0·714	3·57	0·579	2·89	0·135	0·675
„ 27.....	610 to 1000	yellow 1013	acid	0·403	4·03	0·322	3·22	0·081	0·81
„ 28.....	1000	1015	acid	0·799	7·99	0·643	6·43	0·156	1·56
„ 29.....	650 to 1000	1015	acid	0·484	4·48	0·389	3·89	0·095	0·95
„ 30.....	800 to 1000	1016	acid	0·711	7·11	0·601	6·01	0·110	1·10
									Averages Before and
March 22 to 25 (inclusive) (before injection)				0·546	5·46	0·461	4·61	0·085	0·85
„ 26 to 29 („) (after „)				0·600	5·11	0·483	4·11	0·117	0·99

of Dog, 17 Kilogrammes.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other compounds.	P_2O_5.
0·0386	0·386	0·0059	0·059	0·185	1·85	82	18	7·0	1·06	9·94	33
0·0311	0·311	0·0055	0·055	0·158	1·58	86	14	6·2	1·08	6·72	31
0·0330	0·330	0·0069	0·069	0·187	1·87	84	16	5·6	1·17	9·23	32
0·0361	0·361	0·0064	0·064	0·154	1·54	86	14	6·7	1·18	6·12	28
venom injected at 11 A.M.											
0·0434	0·217	0·0053	0·0265	0·237	1·185	81	19	6·1	0·75	12·15	33
0·0325	0·325	0·0130	0·130	0·130	1·30	80	20	8·1	3·23	8·67	32
0·0462	0·462	0·0087	0·087	0·234	2·34	80	20	5·8	1·09	13·11	29
0·0325	0·325	0·0073	0·073	0·141	1·41	81	19	6·7	1·50	10·8	29
0·0448	0·448	0·0102	0·102	0·180	1·80	85	15	6·3	1·44	7·26	25
After the Injection.											
0·0347	0·347	0·0062	0·062	0·171	1·71	84	16	6·4	1·12	8·0	31
0·0386	0·332	0·0086	0·079	0·185	1·56	81	19	6·7	1·64	11·18	31

Table

Date.	Quantity of urine in c.c.	Sp. gr.	Reaction.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.
				20 milligrammes Cobra venom (two minimum					
1904— April 8	No urine	passed.							
„ 9	760 to 1000	1015	acid	0·603	6·03	0·518	5·18	0·085	0·85
„ 10	690 to 1000	1015	acid	0·483	4·83	0·409	4·09	0·074	0·74
„ 11	500 to 1000	1015	acid	0·440	4·40	0·372	3·72	0·068	0·68
				Averages after					
April 9, 10, and 11				0·509	5·09	0·433	4·33	0·076	0·76

The minimum lethal dose of the venom as determined is 0·00025 gramme per kilogramme of body weight. Aseptic precautions were used. The effects of this injection were studied for five days and a second injection of 2·5 milligrammes Cobra venom dissolved in 1 c.c. saline solution was injected on the 25th at 9.30 A.M. and the examination of the urine carried on till November 30.

Experiment 2.

For this experiment a collie, weighing 17 kilogrammes, was used. The same precautions were taken and the same amount of food given.

The analyses of the urine were begun on March 22, 1904, and on the 26th 5 milligrammes Cobra venom in 0·5 c.c. sterilised normal saline solution were injected subcutaneously in the side at 11 A.M. A few hours after the injection the dog vomited, but there was no other symptom. The effects of the venom were studied till the 30th.

III.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other compounds.	P_2O_5.
lethal doses) injected at 1 P.M. on April 8.											
0·0408	0·408	0·0084	0·084	0·104	1·04	86	14	6·8	1·39	5·81	17
0·0314	0·314	0·0076	0·076	0·111	1·11	85	15	6·5	1·57	6·93	23
0·0316	0·316	0·0066	0·066	0·124	1·24	85	15	7·2	1·50	6·30	28
the injection.											
0·0346	0·346	0·0075	0·075	0·113	1·13	85	15	6·8	1·49	6·35	23

Experiment 3.

The same collie was used for this experiment. On April 8, at 1 P.M., 20 milligrammes Cobra venom in 2 c.c. sterilised normal saline solution were injected subcutaneously. This amount is equivalent to two minimum lethal doses. No urine was passed on the 8th. The urine of the 9th, 10th, and 11th was examined. On the 11th an abscess was observed below the seat of inoculation and the animal was destroyed by chloroform.

Experiment 4.

For this experiment a retriever, weighing 12 kilogrammes, was used. The analyses were begun on June 30 and were continued till July 12. On the 6th, 7th, and 8th July 3·3 milligrammes Cobra venom were injected subcutaneously each day at 3 P.M.

Table IV.—Weight

Date.	Quantity of urine in c.c.	Sp. gr.	Reaction.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.
1904—									
June 30	600 diluted to 1000	1012	acid	0·369	3·69	0·311	3·11	0·058	0·58
July 1	350 to 500	1016	acid	0·515	2·576	0·423	2·11	0·092	0·46
„ 2	600 to 1000	1013	acid	0·386	3·86	0·322	3·22	0·064	0·64
„ 3	440 to 500	1015	acid	0·605	3·02	0·526	2·63	0·079	0·39
„ 4	600 to 1000	1010	acid	0·274	2·74	0·249	2·49	0·025	0·25
„ 5	500	1015	acid	0·658	3·29	0·526	2·63	0·132	0·66
„ 6*	800 to 1000	1010	acid	0·288	2·88	0·218	2·18	0·070	0·70
„ 7*	270 to 500	1030	acid	0·470	2·35	0·342	1·81	0·128	0·54
„ 8*	100 to 500	1048	acid	0·595	2·97	0·479	2·39	0·116	0·58
„ 9	200 to 500	1030	acid	0·616	3·08	0·489	2·44	0·127	0·64
„ 10	500	1013	acid	0·552	2·76	0·426	2·13	0·126	0·63
„ 11	500	1013	acid	0·655	3·27	0·496	2·48	0·159	0·795
„ 12	600	1012	acid	0·535	3·11	0·445	2·67	0·090	0·538
				Averages of six days before					
June 30, July 1, 2, 3, 4, and 5 (before injections).				0·468	3·197	0·393	2·52	0·075	0·49
July 6, 7, 8, 9, 10, and 11 ..... (after „).				0·529	2·88	0·42	2·24	0·121	0·65

* On July 6, 7, and 8, Cobra venom 3·3 milligrammes injected each day.

of Dog, 12 Kilogrammes.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other compounds.	P_2O_5.
0·0244	0·244	0·0042	0·042	0·132	1·32	84	16	6·6	1·14	8·26	33
0·0468	0·234	0·0062	0·031	0·201	1·005	82	18	9·0	1·20	9·80	39
0·0417	0·417	0·0052	0·052	0·162	1·62	83	17	10·8	1·34	4·46	42
0·0582	0·291	0·0070	0·035	0·180	0·90	87	13	9·6	1·16	2·24	30
0·0238	0·238	0·0050	0·050	0·106	1·06	90	10	8·7	1·84	0·0	39
0·0560	0·280	0·0099	0·0497	0·280	1·40	80	20	8·5	1·43	10·07	43
0·0375	0·375	0·0039	0·039	0·120	1·20	76	24	13·0	1·36	9·64	42
0·0375	0·1875	0·0045	0·0225	0·206	1·03	73	27	8·0	1·04	17·96	44
0·0465	0·2325	0·00728	0·0364	0·250	1·25	80	20	7·8	1·22	10·98	42
0·0538	0·2690	0·00938	0·0469	0·270	1·35	79	21	8·7	1·52	10·78	44
0·0566	0·2828	0·01288	0·0644	0·228	1·14	77	23	12·5	2·33	8·17	41
0·0582	0·2910	0·01256	0·0628	0·243	1·21	76	24	8·9	1·92	13·8	37
0·0557	0·3343	0·01008	0·0604	0·189	1·13	83	17	14·0	1·90	1·1	35
and six days after the injection.											
0·0418	0·2840	0·00625	0·0433	0·177	1·217	84	16	8·9	1·35	5·8	38
0·0483	0·2730	0·0086	0·0453	0·219	1·19	77	23	9·8	1·56	11·79	42

General Results.

No albumin was ever passed as the results of any injections.

	Experiments.							
	I.		II.		III.		IV.	
	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.
Averages before injection of venom	0·919	4·59	0·546	5·46	0·546	5·46	4·68	3·197
Averages after 1st injection ...	0·954	4·77	0·600	5·11	—	—	—	—
Averages after 2nd injection ...	0·751	4·22	—	—	0·509	5·09	—	—
After three injections							5·29	2·88

In no case was there any indication of an increased proteid metabolism.

Distribution of Nitrogen in Urea and Other Compounds.

Percentages of Urea Nitrogen to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection	80	84	84	84
„ after first injection.....	80	81	—	—
„ „ second injection...	78	—	85	—
After three injections.....				77

In three of the experiments there was a slight fall in the production of urea nitrogen, most marked in Experiment IV where three doses in succession had been given.

Percentages of Ammonia Nitrogen to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection.....	5·57	6·4	6·4	8·9
„ after first injection	7·99	6·7	—	—
„ „ second injection ...	11·6	—	6·8	—
After three injections.....				9·8

In Experiment I there was a marked rise and in Experiment IV a slight rise, while the changes in the other two were in the same direction.

Percentages of Nitrogen in Purin Bodies to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection.....	0·85	1·12	1·12	1·35
„ after first injection	1·18	1·64	—	—
„ „ second injection ...	1·16	—	1·49	—
After three injections.....				1·56

These changes show a slight rise in each experiment.

Percentages of Nitrogen in Other Compounds to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection.....	13·91	8·11	8·11	5·8
„ after first injection	10·58	11·18	—	—
„ „ second injection ...	8·89	—	6·35	—
After three injections.....				11·78

In two experiments there was a rise and in two a fall.

Percentages of P_2O_5 to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection	23	31	31	38
„ after first injection.....	29	31	—	—
„ „ second injection ...	28	—	23	—
After three injections.....				42

In two experiments a rise, in one no change, and in one a fall. The variations in the P_2O_5 do not correspond to the changes in the purin bodies.

Conclusions.

1. Practically no change in rate of proteid metabolism was induced by the administration, in spite of well marked local reaction.

2. A slight decrease in the proportion of urea nitrogen, quite insignificant compared with that produced by diphtheria toxine and various drugs, was observed.

3. A slight rise in the proportion of ammonia nitrogen occurred.

4. There was a slight rise in the proportion of nitrogen in purin bodies.

5. The nitrogen in other compounds showed no constant change.

6. The P_2O_5 excreted showed no constant change, but in two experiments there was a slight rise.

The change produced in the proteid metabolism is, therefore, small, and such as it is, being in the directions of decreased elaboration of urea and increase in the proportion of nitrogen excreted as ammonia, it seems to indicate a slight toxic action on the hepatic metabolism rather than a general action on the proteid changes; and tends to confirm the view that the poison acts chiefly upon the nervous system.